



TECHNICAL MANUAL

Copper (Cu) Colorimetric Assay Kit

- **SKU CODE:** MAES0175
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

1. Assay summary

- Standard tube preparation
- Sample tube preparation
- Add chromogenic agent
- Incubate at 37°C for 5 min
- Measure OD value at 580 nm

2. Intended use

This kit can be used to measure copper (Cu) concentration in serum, plasma, and animal tissue samples.

3. Detection principle

Under acidic conditions, copper ions in the sample react with 3,5-DiBr-PAESA to form a purple complex with a maximum absorption peak at 580 nm. The copper ion content can be calculated indirectly by measuring the OD value at 580 nm.

4. Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500 Assays)	Storage
Reagent 1	Chromogenic Agent A	17.5 mL x 1 vial	35 mL x 1 vial	44 mL x 4 vials	2-8°C, 12 months, shading light
Reagent 2	Chromogenic Agent B	Powder x 1 vial	Powder x 2 vials	Powder x 10 vials	2-8°C, 12 months, shading light
Reagent 3	100 µmol/L Copper Standard	1 mL x 1 vial	1 mL x 1 vial	5 mL x 1 vial	2-8°C, 12 months
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (575-585 nm), Vortex mixer, Micropipette, Water bath

Reagents:

Double distilled water

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For small volume reagents, please centrifuge before use to obtain sufficient amounts of reagents.

6. Reagent preparation

- 1. Preparation of chromogenic agent B working solution:** Dissolve one vial of chromogenic agent B with 1.25 mL double distilled water and mix thoroughly. Aliquot and store at -20°C for 1 month.
- 2. Preparation of chromogenic agent:** For each tube, prepare 300 µL of chromogenic agent by thoroughly mixing 280 µL of chromogenic agent A and 20 µL of chromogenic agent B working solution. The chromogenic agent should be prepared fresh immediately before use.
- 3. Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 100 µmol/L copper standard with double distilled water to create a serial concentration series. The recommended dilution gradient is: 0, 5, 10, 20, 30, 40, 50, 60 µmol/L.

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, serum or plasma can be stored at -80°C for one month.

Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation: 20 mg).
2. Wash tissue in cold PBS (0.01 M, pH 7.4).
3. Homogenize 20 mg tissue in 180 µL double distilled water with a dounce homogenizer at 4°C.
4. Centrifuge at 10,000 × g for 10 minutes to remove insoluble material. Collect supernatant and keep on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

The recommended dilution factor for different samples is as follows (for reference only):

Human serum: 1

Human plasma: 1

Dog serum: 1

Rat serum: 1

Rabbit serum: 1

Porcine serum: 1

Note: The diluent is double distilled water. For dilution of other sample types, please perform a pre-test to confirm the dilution factor.

8. The key points of the assay

1. Prevent the formation of bubbles in the microplate, as this will affect the OD value measurement.
2. Serum and plasma samples should be clarified and avoid hemolysis. Heparin is recommended as anticoagulant.

9. Operating steps

1. Standard tube: Add 15 μ L of standard solution with different concentrations into tubes. Sample tube: Add 15 μ L of test sample into tubes.
2. Add 230 μ L of chromogenic agent into each tube.
3. Cover the plate with sealer and incubate at 37°C for 5 minutes.
4. Measure the OD value at 580 nm with microplate reader.

10. Calculation

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean OD value of the blank (Standard #1) from all standard readings. This is the absolute OD value.
3. Plot the standard curve using absolute OD value of standards and corresponding concentration as y-axis and x-axis respectively. Create the standard curve ($y = ax + b$) with graph software (or Excel).

The sample:

1. Serum (plasma) sample:

$$\text{Cu content } (\mu\text{mol/L}) = (\Delta A - b) \div a \times f$$

2. Tissue sample:

$$\text{Cu content } (\mu\text{mol/g}_{\text{prot}}) = (\Delta A - b) \div a \times f \div C_{\text{pr}}$$

[Note]

f: Dilution factor of sample before test

C_{pr}: Concentration of protein in sample (g_{prot}/L)

ΔA: Absolute OD (OD_{Sample} - OD_{Blank})

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	4.80	26.70	52.00
%CV	3.5	3.0	2.5

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	4.80	26.70	52.00
%CV	2.8	3.2	3.3

Recovery

Three samples of high, middle, and low concentrations were tested in parallel 6 times each to achieve an average recovery rate of 105%.

Recovery

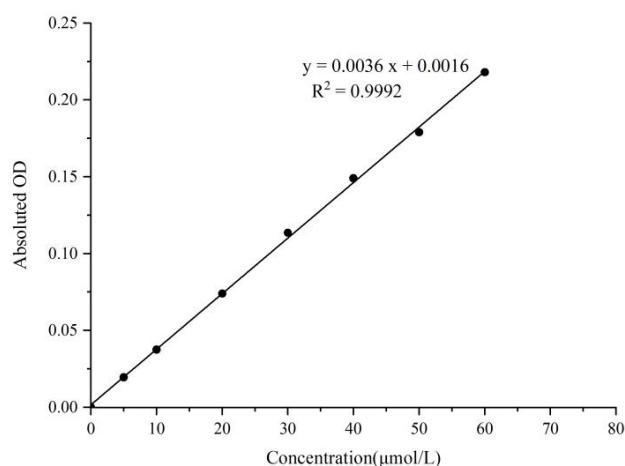
Standard 1	Standard 2	Standard 3
7.5	28.5	48.2
7.6	30.5	51.6
101	107	107

Sensitivity

The analytical sensitivity of the assay is 1.84 $\mu\text{mol/L}$. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve data

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, or temperature effects), the standard curve and data are provided below for reference only:



Standard curve data

Concentration (μmol/L)	Average OD	Absolute OD
0	0.102	0
5	0.122	0.020
10	0.147	0.046
20	0.196	0.094
30	0.241	0.139
40	0.281	0.179
50	0.331	0.230
60	0.365	0.264

12. Appendix II Example Analysis

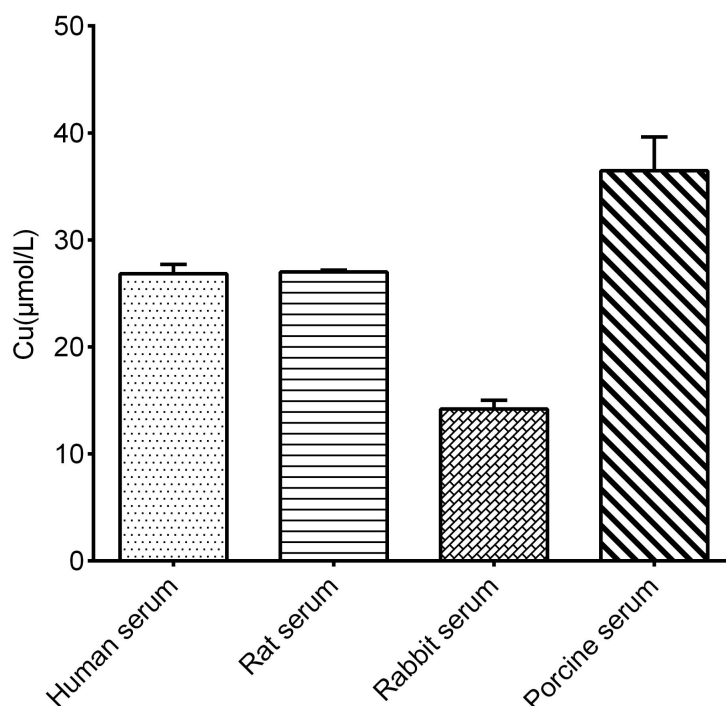
Example analysis:

Take 15 μL of rat serum and carry out the assay according to the operating steps. The results are as follows:

Standard curve: $y = 0.0036x + 0.0016$, the average OD value of the sample well is 0.170, the average OD value of the blank well is 0.074, and the calculation result is:

Cu content (μmol/L) = $(0.170 - 0.074 - 0.0016) \div 0.0036 = 26.22 \mu\text{mol/L}$

Detection of human serum, rat serum, rabbit serum, and porcine serum according to the protocol shows the following results:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.



Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.